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Extracting sand microarthropods: a carbon tetrachloride flotation method

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Abstract

A carbon tetrachloride flotation method is tested in the laboratory and in the field. Its efficiency approaches 100 % in the laboratory and densities observed in the field exceed all densities previously published. The method is simple to perform but limited to low organic soils.

Keywords: Sand, microarthropods, extraction.

L'extraction des microarthropodes des sables: une méthode de flottation au tétrachlorure de carbone.

Résumé

Une méthode de flottation au tétrachlorure de carbone est testée en laboratoire et sur le terrain. Son efficacité approche les 100 % en laboratoire et les densités observées sur le terrain dépassent toutes les densités publiées auparavant. La méthode est simple mais limitée à des sols à faible teneur organique.

Mots-clés : Sable, microarthropodes, extraction.

INTRODUCTION

Little detailed information is available on the ground fauna of coastal sand dunes (Wallwork, 1976). This applies also to other sandy habitats such as the Namib desert. Even if dune sandy soils are, in comparison to forest soils, much simpler in structure and much poorer in organic content, the sand dune fauna appears to be highly specialized (Coineau and Massoud, 1977; Coineau *et al.*, 1978; Coineau and Seely, 1983). The soil fauna of sand dune is dominated by arthropods (Wallwork, 1976) among which numerous species adapted to the sand have been described. Collembola have been described from sandy habitats by Petersen (1965), Massoud and Thibaud (1985), Thibaud and Massoud (1988), Thibaud and Christian (1989); endeostigmatid mites, by Strenzke (1954), Coineau *et al.* (1967), Coineau and Théron (1983); gamasid mites, by Willmann (1935, 1951). Various sampling campaigns however revealed that a great deal of the arthropod fauna was still undescribed and that little ecological information was available. We have virtually no information on the number of arthropod species coexisting in sand dunes or on their densities. Poinsot (1966) stated that collembolan populations were severely reduced in the dune system at Beynes, Haute Camargue, France and that collembolans were living in the upper two cm, at most in the upper 10 cm. Collembolan densities in sand dunes are pretty low and vary from 0.5 to 28 individuals/l (Thibaud and Christian, 1989) and from 6 to 144 individuals/l (Christian and Thibaud, 1988). Up to 11 arthropods/m^2 were caught by Watson (1987) in two Namib dune habitats.

Such low densities and the lack of biological data on the sand dune fauna may probably be attributed to the extraction methods used to explore this environment. Watson (1987) sieved sand to collect fishmoths from dune slipfaces in the Namib desert. Sand sieving is probably useful to collect large arthropods but is certainly useless to catch microarthropods.

Even if the Berlese Tullgren extraction was used by Petersen (1965) and Poinsot (1965), this method turns out to be of little interest: sand falls through the sieve when the sample dries and the efficiency has never been evaluated.

Another method, the grease film extraction designed by Aucamp and Ryke (1964), was modified and used by Coineau and Seely (1983) to collect microarthropods from sandy soils. Psammophilous Collembola were caught through sand washing by Thibaud and Christian (1989). In both cases, the sampling units were very large (1 or several liters) and densities observed were low.

Roughly, extraction methods can be divided into active and passive (or mechanical) methods. The most commonly used active technique is the Berlese Tullgren funnel. Passive techniques include hydrocarbon adhesion methods such as that used by Coineau and Seely (1983), washing methods used by Thibaud and Christian (1989) and flotation methods. Flotation methods rely primarily on differences in specific gravity or in sedimentation rates to separate animals from soil particles. The most common approach is to use an extracting solution (usually sugar or a salt in water) with a specific gravity sufficient to suspend the animals but which allows soil particles to settle out (McSorley and Walter, 1991).

Flotation techniques were tested as they may be simple to use, quick and provide, given the low organic content of sandy soils, samples with no or few detritus.

MATERIALS AND METHODS

As stated in the introduction, flotation methods rely primarily on differences in specific gravity to separate animals from soil particles. The most common extracting solutions used to extract microarthropods are sugar or salt in water. A detailed list of solutions used for flotation of animals in soil is given by Murphy (1962: table 23). A concentrated solution of sodium chloride was already used to extract soil nematodes by Robertson (1929) and was "rediscovered" recently by Fain and Hart (1986) and Hart and Fain (1987) to extract mites. Compared to other applications of the NaCl solution (de Gridelet-Saint Georges, 1976; Bronswijk *et al.*, 1978), the novelty in Fain's technique was to initially soak the sample in 80 % ethanol before suspending the mites in the salt solution, so that mite density is lowered to that of ethanol, i.e. 0.86. However, the specific gravity of NaCl saturated water solution is only 1.2 and we tested two other saturated solutions, calcium chloride and potassium carbonate. Properties of these solutions are given in table 1.

Table I. Properties of products tested as flotation media.

Name	Formula	ρ (1)	h (1)	b.p. (1)	Toxicity	Price (2)
Water	H_2O	0,9982	1,00	100,0		
Ethanol	C_2H_5OH	0,7893	1,20	78,5		
SURSATURATED AQUEOUS SOLUTIONS						
Sodium chloride	NaCl	1,1972	1,98			401 BEF/kg
Calcium chloride	$CaCl_2$	1,3957	8,96			529 BEF/kg
Potassium carbonate	K_2CO_3	1,5404	9,34			499 BEF/kg
ORGANIC COMPOUNDS						
Carbon tetrachloride	CCl_4	1,5940	0,97	76,74	yes	491 BEF/l
Tetrachloroethene (*)	$Cl_2C{:}CCl_2$	1,6227	?	121	yes	592 BEF/l

ρ : density at 20 °C
h : viscosity (in cp) at 20 °C
b.p. : boiling point in °C

(*) Used by Solomon (1962) but not tested here.

(1) From Weast (1973). (2) From the Aldrich Catalogue, 1992 Belgian edition.

Organic solvents have also been used as flotation media for soil animals: carbon tetrachloride-petroleum ether mixture, chloroform, dichloroethylene, etc. Considering also the price, we restricted our tests to carbon tetrachloride, a solvent used in medical acarology by Oshima (1970).

In the laboratory

Few methods have been tested in the laboratory. Exceptions are Sinha (1964), Bronswijk (1973), Hart and Fain (1987) and Walter *et al.* (1987). To test the efficiency of different methods batches of 15 arthropod specimens were prepared and mixed with sand. For each test (combination species x product), there were three replicates, each with 15 individuals. All samples were soaked in 80 % ethanol for several days as recommended by Hart and Fain (1987). Samples were then poured into an Erlenmeyer containing the medium to test and stirred thoroughly for at least 5' using a magnetic stirrer. Supernatant was then controlled and removed with a Pasteur micropipette at regular intervals.

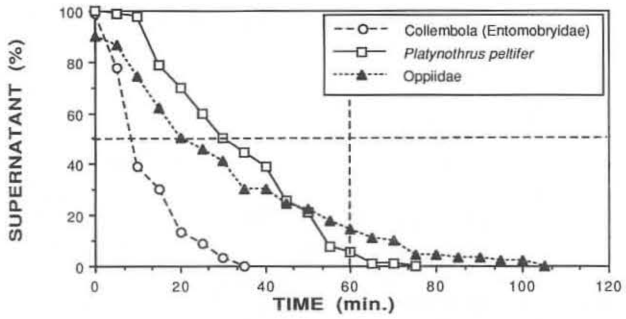

Figure 1. – Flotation duration of different taxa in NaCl saturated water (Fain's technique). Each point represents the mean value of three batches of 15 individuals.

The species

Three groups of species were tested with NaCl saturated water: one Collembola Entomobryidae (mostly *Entomobrya nivalis*), and two Oribatida, a large species, *Plathynothrus peltifer* and small mites of the family Oppiidae (most specimens were *Oppiella nova*).

The products

The efficiency of different flotation media were tested using Oppiidae. All products tested are listed in table I. Different NaCl solutions were tested (densities 1.10, 1.15 and 1.20).

The sample volume

500 ml and 1 000 ml Erlenmeyers were tested. Different sand volumes were also tested and the substrate thickness at the bottom of the Erlenmeyer was measured. To test the efficiency of the stirring, these samples were manually agitated after the first control and the supernatant analyzed again.

In the field

The sand fauna was studied to the field in Carnon, near Montpellier (France). The fauna was extracted

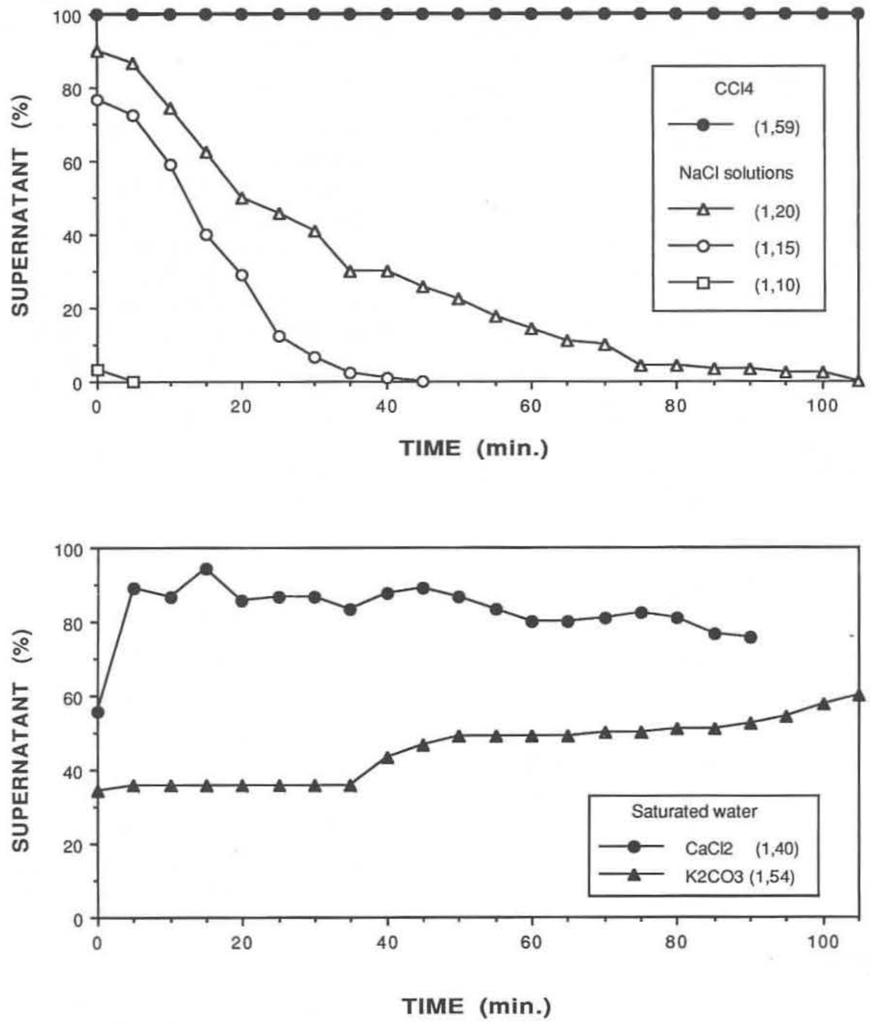

Figure 2. – Flotation duration of Oppiidae in different flotation media. Media densities are given between brackets. Each point represents the mean value of three batches of 15 individuals.

from the fore dune along the Mediterranean coast. Eleven 48 cm^3 samples were collected at different depths (from − 10 to − 70 cm). Two series of four samples were collected under marram grass and a third series of three under sand with no vegetation at all.

The samples were brought back to the laboratory and immersed into 80 % ethanol on the same day. Each sample was poured into a 1 000 ml Erlenmeyer filled up with CCl_4 and stirred thoroughly for at least 5' using a magnetic stirrer. Supernatant was then examined under a dissecting microscope, and animals counted and collected with a Pasteur micropipette. The sample was stirred again until there were no more animals floating at the surface.

RESULTS

In the laboratory

The species

The three groups of species tested show a similar behavior when treated in NaCl saturated water (Fain's technique). At starting, all specimens – or nearly all in the case of Oppiidae – float at the surface (fig. 1). However after half an hour, fifty percent of the specimens sink. The softest the animal integument, the fastest they sink.

The products

Figure 2 compares the efficiency of different flotation media to extract Oppiidae from the sand samples. The efficiency of NaCl aqueous solutions decreases quickly with density. Satisfactory results are obtained only with saturated water but animals sink as earlier mentioned. Surprisingly, the other aqueous solutions yield poor results although they have a high specific density. In fact, many animals adhered to the inner side of the Erlenmeyer or, if they did not, remounted very slowly to the surface. This is explained by the high viscosity of these solutions (*see* table I) which were nearly syrupy. The best results were obtained with carbon tetrachloride: hundred percent of animals float and remain at the surface for hours.

The sample volume

Samples of ca 50 cm^3 may be treated as a whole when 1 000 ml Erlenmeyer are used. As a matter of fact, the problem is less the sample volume itself than the substrate thickness at the bottom of the Erlenmeyer. Once the sand thickness exceeds 30 mm, the stirring becomes inefficient as shown in figure 3.

In the field

A total of 599 arthropods belonging to 31 species was collected. Most of them belonged to Collembola and Actinedida, especially to Endeostigmata. To extract all specimens from a sample, it was necessary to repeat the cycle stirring-examination-collecting up to ten times, which was very time consuming (nearly one half day). Most of them were below 200 µm long (fig. 4). The most populated sample contained 120 specimens (ca 2 495 individuals/liter), the least, only five specimens (ca 104 individuals/l) and up to 20 species were found to coexist in one sample. Under marram grass, Collembola densities varied from 125 to 790 individuals/liter, i.e. 5 to 10 times highest densities published in the literature (*see* the introduction).

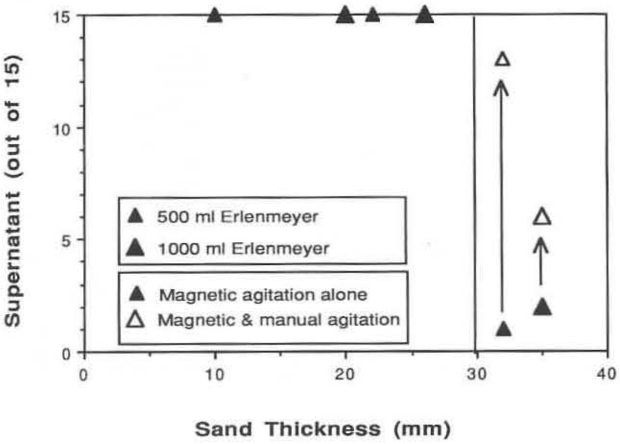

Figure 3. – Percentage of *Plathynothrus peltifer* found in carbon tetrachloride supernatant with two types of Erlenmeyer and for different sand thicknesses at the bottom of the Erlenmeyer. When magnetic agitation alone was not sufficient to regain all mites, samples were agitated with a glass rod and the supernatant analyzed again. Each point represents 15 individuals.

The density expressed in individuals/m^2 was found to be much higher than expected: 175 000 under sand with no vegetation, and from 550 000 to 1 400 000 under marram grass. These results are really amazing given the low organic content of sand. A detailed account of the fauna is given by André *et al.* (1993).

CONCLUSIONS

Some flotation media must be rejected as they are poorly efficient in extracting arthropods. This is the case of calcium chloride and potassium carbonate saturated solutions which are too viscous. Fain's technique is also little reliable: after half an hour, most animals sink, especially those which are small and soft-bodied such as Actinedida. Yet, the counting of microarthropods may require several hours for the most populated samples.

The only product which give acceptable results is carbon tetrachloride. Its specific density is high, and the viscosity is low. No special deformation of animals has been noticed. The product is easy to buy and cheap. The major drawback is its toxicity, working under hood seems to be a prerequisite. Tetrachloroethene would probably give similar results but it is more expensive and probably as toxic (table I).

The flotation method itself is simple to perform. It requires easily available material (carbon tetrachloride, 1 000 ml Erlenmeyer, magnetic stirrer). It was found

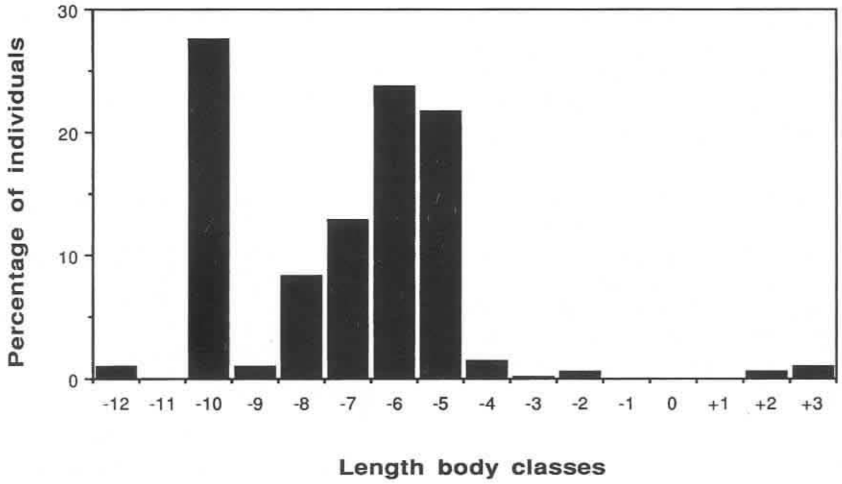

Figure 4. – Distribution of the number of individuals per body length. The body length classes used follow Morse *et al.* (1988).

to be time-consuming but this is due mainly to the size of the sand fauna: collecting and counting tiny mites of 100-200 μm is exhausting whatever the extraction method. It provides clean samples, which is a major concern with so small arthropods. Eggs and pupating animals are also recovered, as well as dead arthropods, which may be considered as a drawback.

Laboratory tests show that its efficiency approaches 100 % with carbon tetrachloride: losses may occur when animals are transferred, either if one tries to decant the Erlenmeyer supernatant, or when animals are taken with a pipette or when they are mounted for microscopic examination. On the other hand, densities of microarthropods collected in the field exceed all densities previously published and clearly demonstrate the high efficiency of the method.

The major shortcoming of the method is that it can apply only to low organic content soil such as sand. With litter for example, microarthropods would float in a morass of organic detritus, which would make their counting almost impossible. Nevertheless, the method has a wide range of application. Sandy soils represent an important part of the African soils, from the regosols and xerosols encountered in northern Africa to the arenosols observed in the Kalahari (ca 42 000 km^2). They are found in environments as contrasted as the dunes of Namib and the "Niayes" of Sénégal. Moreover, the method might probably apply to the extraction of the euedaphic fauna which colonizes the deepest horizons of soils and remains largely unknown as already stressed by Lebrun and Wauthy (1981).

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